SUPPLEMENTAL MATERIAL

Maternal cholestasis during pregnancy programs metabolic disease in offspring

Supplemental Tables

Supplemental Table 1: Morphometric features of mouse cholestatic pregnancy

Maternal diet	Litter size	Fetal body weight (g)
NC	7.2 ± 1.4	0.83 ± 0.2
CA	6.5 ± 1.2	0.76 ± 0.2

NC: normal chow, CA: cholic acid; n=17 animals per group; values represent the mean ± SEM

Supplemental Table 2: Transcriptomic profile in livers of western diet (WD)-fed females from cholestatic mothers as predicted with Ingenuity Pathway Analysis of microarray data.

Gene ID	Gene name	fold-change	Pathway
BC006872	*Cel*	169	Fatty acid synthesis and cleavage
AK133952	*Myc*	169	Fatty acid synthesis and cleavage
AK171155	*Dpep2*	66	Fatty acid synthesis and cleavage
BC145908	*Pla2g1b*	42	Fatty acid synthesis and cleavage
AK152189	*Il6*	2.5	Fatty acid synthesis and cleavage
BC027742	*Clec7a*	2.4	Fatty acid synthesis and cleavage
AF359558	*Il4*	1.9	Fatty acid synthesis and cleavage
AK079235	*Ggt1*	1.9	Fatty acid synthesis and cleavage
BC094923	*Pnliprp1*	1.6	Fatty acid synthesis and cleavage
BC062902	*Gpc1*	1.5	Fatty acid synthesis and cleavage
BC145867	*Ccl2*	1.5	Fatty acid synthesis and cleavage
U49110	*Lepr*	-1.5	Fatty acid synthesis and cleavage
AB005909	*Dmbt1*	386	Inflammation
BC119057	*Mmp7*	9	Inflammation
AK171435	*Mmp12*	3.6	Inflammation
AK007645	*Gal3st1*	3.6	Inflammation
BC003780	*Chi3l1*	3	Inflammation
BC132069	*Lcn2*	2.7	Inflammation
BC061154	*Chi3l3*	2.4	Inflammation
BC061126	*Ccl7*	2.3	Inflammation
BC141556	*Il8ra*	2.3	Inflammation
BC002063	*Lgals1*	2.2	Inflammation
AF359558	*Il4*	2	Inflammation
BC006783	*Ctgf*	1.9	Inflammation
BC003480	*Capg*	1.8	Inflammation
BC010726	*Pla2g7*	1.6	Inflammation
BC033485	*Trem2*	1.6	Inflammation
X16834	*Lgals3*	1.6	Inflammation
BC132022	*Raet1b*	1.6	Inflammation
BC146516	*Ear10*	1.5	Inflammation
AK152189	*Il6*	1.5	Inflammation
BC145867	*Ccl2*	1.5	Inflammation

Fold-change of liver gene expression of WD-fed female offspring from cholestatic mothers compared to hepatic gene expression of WD-fed females from normal mothers. Significant changes ($p \leq 0.05$) with a fold-change ≥ 1.5 are shown. Gene expression profile is consistent with increased fatty acid synthesis and cleavage and inflammation in the WD-fed female from CA-fed mothers.

Supplemental Table 3: Inflammation-related genes that significantly change in white adipose tissue (WAT) of western diet (WD)-fed females from cholestatic mothers as predicted with Ingenuity Pathway Analysis

Gene ID	Gene name	fold-change
AK132915	*Itgad*	15
BC054091	*Serpine1*	4.7
BC055885	*Saa3*	3.5
BC109158	*Selp*	3.2
BC094009	*Krt8*	3.1
BC006783	*Ctgf*	3.1
BC012650	*Cldn3*	3
AK137169	*Itga1*	2.8
BC054530	*Podxl*	2.6
BC096586	*Prlr*	2.6
BC012690	*Vtn*	2.5
AK133483	*Gnaz*	2.4
AK143562	*Lbp*	2.3
BC020530	*Kdr*	2.3
BC007125	*Aqp1*	2.2
BC019460	*Esam*	2.2
AK133933	*Sept4*	2.1
BC062378	*Nos2*	2.1
BC050824	*Tek*	2.1
AK159653	*Itga6*	2
AK169431	*Pecam1*	2
AK028157	*Mrc2*	2
BC020532	*Rapgef3*	2
BC053430	*Pdgfb*	2
BC036175	*Agtr1a*	2
BC065077	*Lhx6*	2
AK172072	*Sh2d3c*	1.9
BC016505	*Ephb4*	1.9
BC004656	*Scarb1*	1.9
AF114266	*Tgm2*	1.9
AK145864	*Lifr*	1.9
BC156151	*Myo10*	1.9
AF469622	*Arap3*	1.9
BC022107	*Cdh2*	1.8
BC075716	*Kit*	1.8
BC021655	*Akr1b3*	1.8
AK140530	*Plcl1*	1.7
S75867	*Itpr1*	1.7
BC021876	*F11R*	1.7
BC156169	*Dock4*	1.7
U28151	*Bcar1*	1.7
BC060129	*Nrp1*	1.5
BC030478	*Mylk*	1.5
AK202858	*Pkd1*	1.5

BC005452	*Bgn*	1.5
BC132544	*Rgs18*	-2.5
AF033112	*Siva1*	-1.6
BC006763	*Cbfb*	-1.5

Fold-change of WAT gene expression of WD-fed female offspring from cholestatic mothers compared to WAT gene expression of WD-fed females from normal mothers. Significant changes ($p \leq 0.05$) with a fold-change ≥ 1.5 are shown. Gene expression profile is consistent with increased inflammation in the WD-fed female from cholic acid (CA)-fed mothers.

Supplemental Table 4: Fetal metabolic pathways in mouse pregnancy

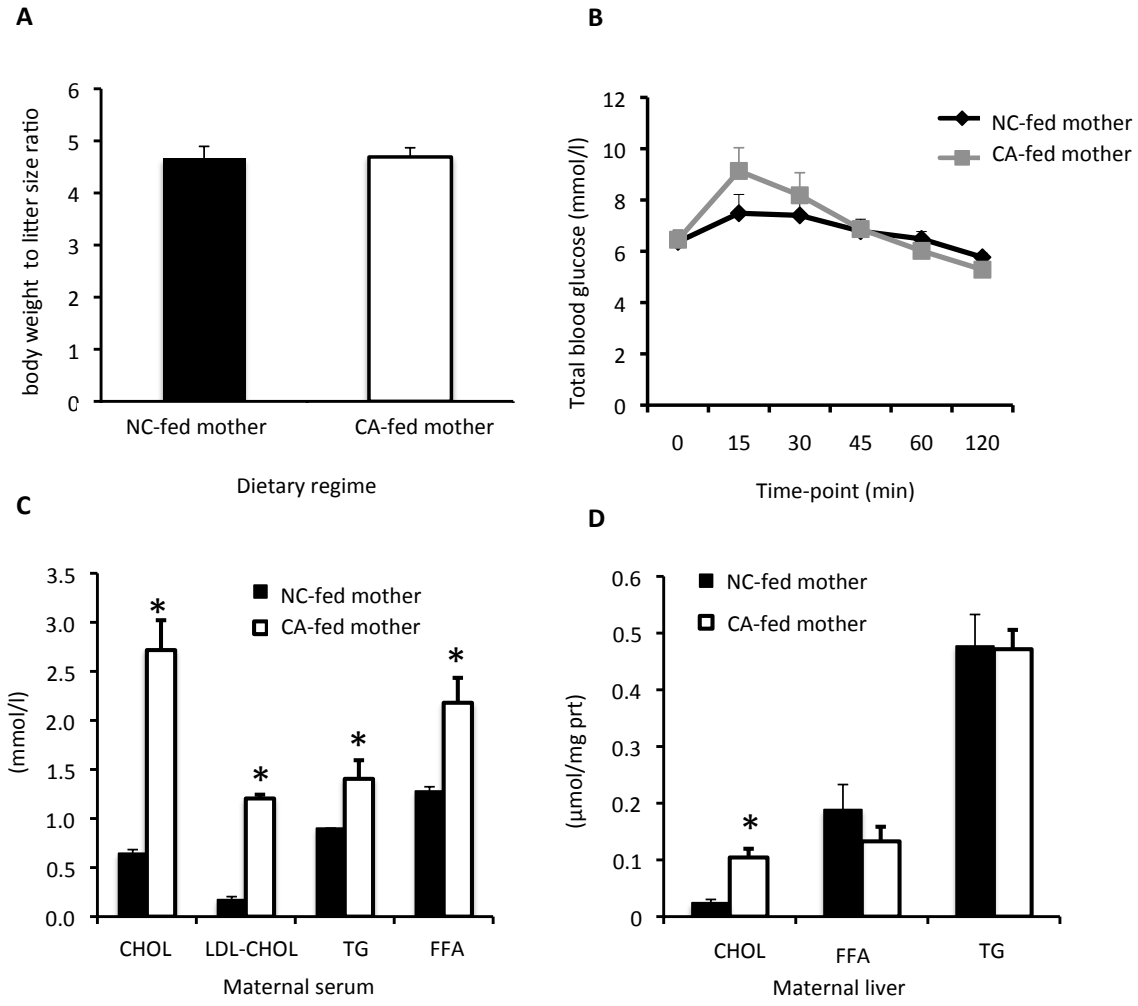
	from NC-fed mother	**from CA-fed mother**
Serum total bile acids (mmol/l)	18 ± 10	72 ± 20*
Bile acid homeostasis pathways (mRNA; relative expression to cyclophilin)		
Bsep	1.15 ± 0.3	5.20 ± 0.8*
Cyp7a1	0.5 ± 0.3	0.3 ± 0.08*
Shp	1.1 ± 0.2	12.6 ± 1.1*
Mrp3	1.2 ± 0.2	3.7 ± 0.5*
Mrp4	1.9 ± 0.4	6.8 ± 1.1*
Sult2a1	1.0 ± 0.1	4.7 ± 0.7*
Hepatic lipid profiles (µmol/mg prt)		
Cholesterol	0.09 ± 0.004	0.13 ± 0.006*
TG	0.29 ± 0.01	0.36 ± 0.02*
FFA	0.13 ± 0.01	0.18 ± 0.03
Cholesterol biosynthesis pathway (mRNA; relative expression to cyclophilin)		
Dhcr-7	0.7 ± 0.09	1.7 ± 0.1*
Hmgcr	1.4 ± 0.3	7.4 ± 0.8*
Srebp2	1.4 ± 0.3	5.8 ± 0.9*
Fatty acid/triglyceride synthesis pathway (mRNA; relative expression to cyclophilin)		
Fas	1.1 ± 0.1	3.5 ± 0.4*
Lxr-α	1.3 ± 0.1	5.7 ± 0.5*
Scd-2	1.0 ± 0.2	2.6 ± 0.6*
Srebp1c	1.3 ± 0.16	3.1 ± 0.2*

The fetuses of cholestatic mothers had increased serum bile acid levels as well as induced bile acid excretory pathways consistent with a cholestatic phenotype (1). Moreover, *de novo* hepatic cholesterol and fatty acid biosynthetic (2) pathways were also increased in fetuses of CA-fed mothers. NC: normal chow, CA: cholic acid; TG: triglycerides, FFA: free fatty acids; n=6 animals per group, * $P \leq 0.05$; values represent mean ±SEM.

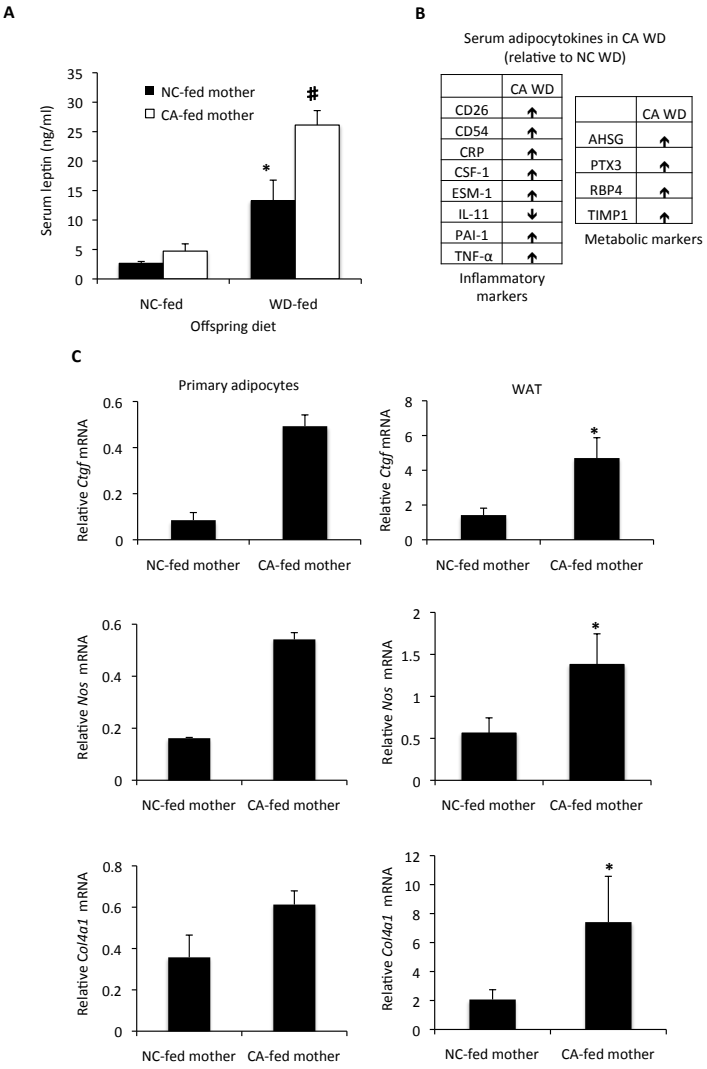
Supplemental Table 5: Primer sequences

Gene name	Forward primer	Reverse primer
	Lipid storage	
Acat-2	ATATGAGCAAGGCTCCTCAC	GGTAGTTGTGAAAGGCATCTG
Adrp (Plin2)	TGGCAGCAGCAGTAGTGGAT	AGCTCACCAAGGGCAGGTT
	Inflammation	
Col4a1	GCCCTTCATTAGCAGCTTTC	GCACTGCGGAATCTGAATG
Ctgf	CAGAACGCACACTGAGGTGA	TGCTATAATTGCCCTCCCCG
Nos	TCAACCTCCTGACTGAAGCA	CCAAGCCATCATTGGGAGTAGA
	Bile acid homeostasis pathways	
Bsep	AAGCTACATCTGCCTTAGACACAGAA	CAATACAGGTCCGACCCTCTCT
Cyp7a1	AGCAACTAAACAACCTGCCAGTACTA	GTCCGGATATTCAAGGATGCA
Shp	CGATCCTCTTCAACCCAGATG	AGGGCTCCAAGACTTCACACA
Mrp3	GCAGCAGAACCAAGCATCAAG	GACCGCATCCTCACCTGG
Mrp4	GGTTGGAATTGTGGGCAGAA	TCGTCCGTGTGCTCATTGAA
Sult2a1	GAAGGCATACCTTTTCCTGCCA	GTAACCAGACACAAGAATATCT
	Cholesterol biosynthesis pathway	
Dhcr-7	GCCAAGACACCACCTGTGACAG	TGGACGCCTCCCACATAACC
Hmgcr	TTGGCACCATGTCAGGCGTCC	AGCGACACACAGGCCGGGAA
Srebp2	CCTAGACCTCGCCAAAGGTG	CAGGCTGTAGCGGATCACAT
	Fatty acid/triglyceride synthesis pathway	
Fas	CCCAGAGGCTTGTGCTGACT	CGAATGTGCTTGGCTTGGT
Lxr-□	AGGAGTGTCGACTTCGCAAA	CTCTTCTTGCCGCTTCAGTTT
Scd-2	AGCGGGCTGCAGAAACTTAG	GGCTGAGTAAGCGCCAGAGAT
Srebp1c	GGAGCCATGGATTGCACATT	GGCCCGGGAAGTCACTGT
	Genes used for normalisation	
Ap2	ACACCGAGATTTCCTTCAAACTG	CCATCTAGGGTTATGATGCTCTTCA
Cyclophilin	TGGAGAGCACCAAGACAGACA	TGCCGGAGTCGACAATGAT

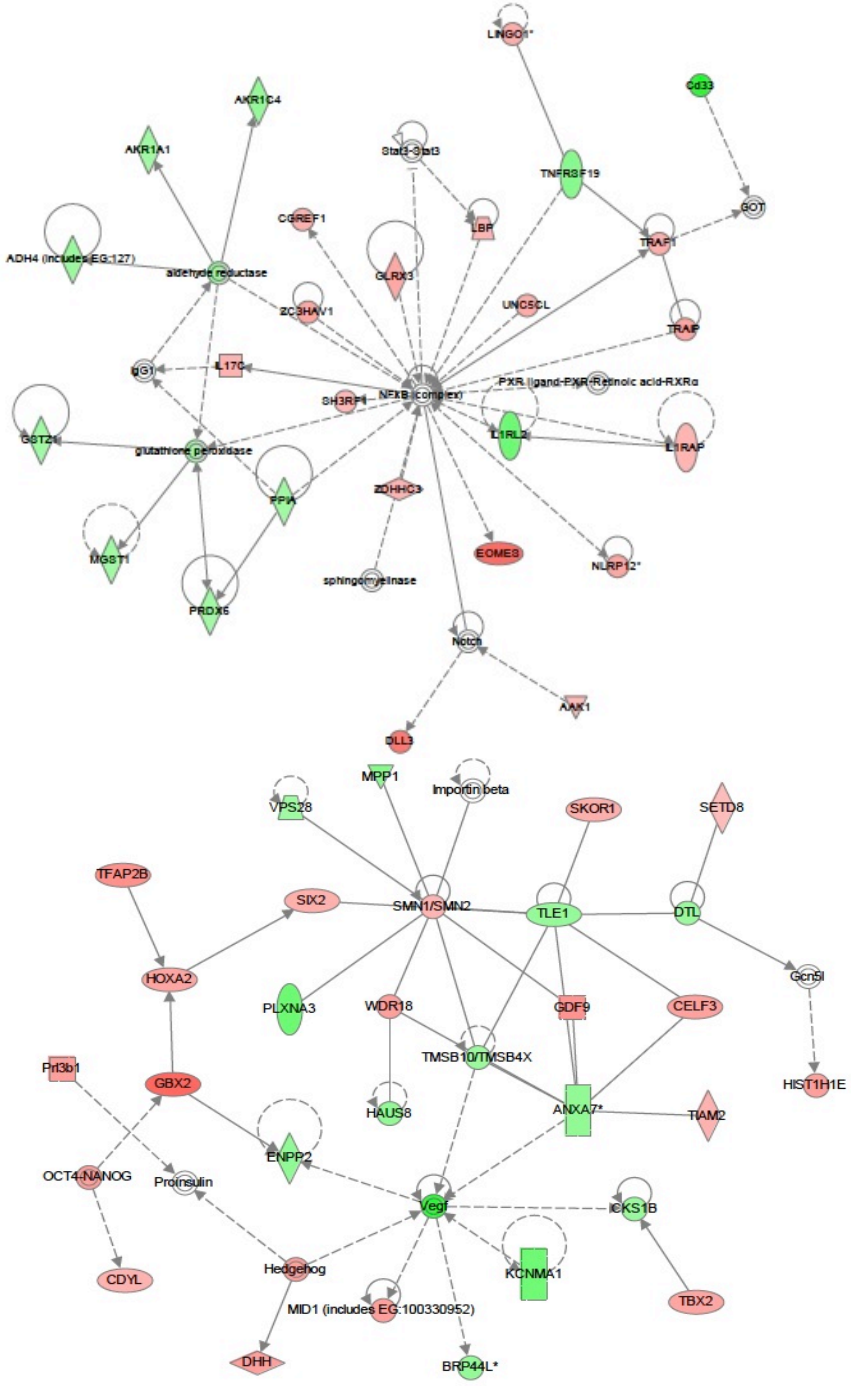
Supplemental Figures



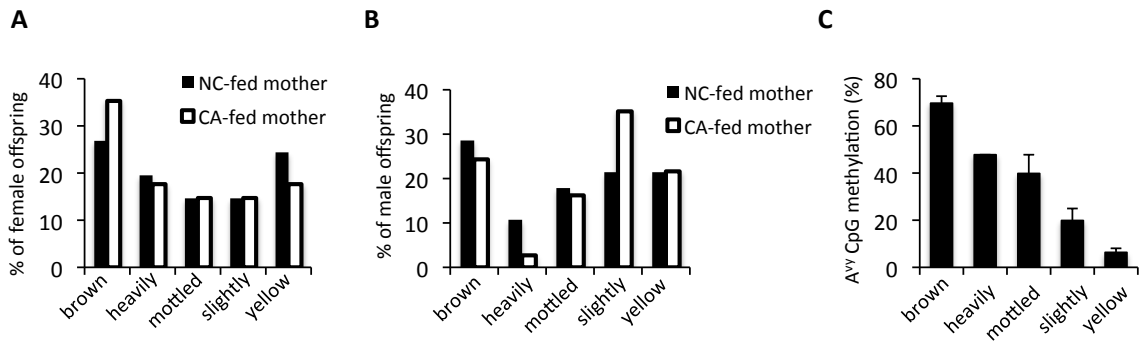
Supplemental Figure 1: Mouse cholestatic pregnancy results in maternal dyslipidemia without affecting body weight and glucose tolerance. A) Body weight to litter size ratio in normal chow (NC)-fed and cholic acid (CA)-fed mice on day 18 of pregnancy, n=6 animals per group. B) Glucose tolerance test in NC-fed and CA-fed mice on day 18 of pregnancy after a 6-hour fast. Following an intraperitoneal injection of glucose (1g/kg body weight), total blood glucose was measured at the indicated time-points, n=6 animals per group. C, D) Lipid levels in NC-fed and CA-fed mice on day 18 of pregnancy in maternal serum (C) and liver (D). Significance of data was established by Student *t*-testing, n=6 animals per group, $*P \leq 0.05$. Chol: cholesterol, LDL-chol: low-density lipoprotein-cholesterol, TG: triglycerides, FFA: free fatty acids. Values represent mean ± SEM.



Supplemental Figure 2: Effects of cholestatic pregnancy on adipose tissue function. A) Serum leptin levels in female offspring after a 4-hour fast, n=6 animals per group, **P* ≤0.05 for differences in offspring fed a different diet, $^{\sharp}$p≤0.05 for differences in offspring exposed to a different intrauterine environment. B) Markers of inflammation and metabolic pathways in serum of WD-fed female offspring of CA-fed mothers (compared to WD-fed offspring of NC-fed mothers). ↑: increase, ↓ decrease. C) *Ctgf*, *Nos* and *Col4a1* mRNA expression relative to *cyclophilin* in differentiated primary adipocytes (left panel) isolated from WD-fed female offspring of NC-fed or CA-fed mothers. Differentiation levels were normalized to the *aP2* differentiation marker. Error bars represent SD of duplicate wells of the same group of animals. WAT tissue of WD-fed offspring (right panel) was also assessed for target gene mRNA levels. n=4 animals per group, **P* <0.05, values represent mean ±SEM. WAT: white adipose tissue, NC: normal chow, CA: cholic acid, WD: western diet, NC WD: WD-fed offspring from NC-fed mothers, CA WD: WD-fed offspring from CA-fed mothers.



Supplemental Figure 3: Altered gene networks in livers of 18-week old normal chow (NC)-fed female offspring that were exposed to bile acids in utero. Networks of genes in livers of female offspring fed NC diet and exposed to high bile acids in utero. Standard: Expression in NC-fed offspring of NC-fed mothers. Green, up-regulated by comparison, and red, down-regulated by comparison. Gene networks were delineated using Ingenuity Pathway Analysis.



Supplemental Figure 4: Cholestatic pregnancy alters the epigenome of 3-week old offspring. A) Coat colour phenotype of female offspring of normal chow (NC)- or cholic acid (CA)-fed mothers. B) Coat colour phenotype of male offspring of NC- or CA-fed mothers. C) Percentage methylation of CpG sites of the A^{vy} cryptic promoter in at least 4 samples per phenotype.

References

1. Goodwin, B., Jones, S.A., Price, R.R., Watson, M.A., McKee, D.D., Moore, L.B., Galardi, C., Wilson, J.G., Lewis, M.C., Roth, M.E., et al. 2000. A regulatory cascade of the nuclear receptors FXR, SHP-1, and LRH-1 represses bile acid biosynthesis. *Mol Cell* 6:517-526.
2. Goldstein, J.L., DeBose-Boyd, R.A., and Brown, M.S. 2006. Protein sensors for membrane sterols. *Cell* 124:35-46.